In the name of Allah the Most Gracious the Most Merciful
Apoptosis and cancer therapy

Presented by: Rostamy, A

Under supervision: Dr. Ahmadpour.

Paramedicine faculty

Qazvin university of medical science
1) Introduction .................... (4-33)
   a) Morphological change ...... (6-10)
   b) Biochemical change ........ (11-16)
   c) extrinsic .................... (20-24)
   d) intrinsic .................... (24-34)

2) Discussion ....................... (33-69)
   a) Oxidative stress ..........
   b) p53 .........................
   c) bcl2 ........................
   d) trail/rhApo2L .............

3) Result .......................... (69-72)

4) References ....................


Apoptosis and cancer therapy

number of article

Source: google scholar
Last updated: 2017/4/14
The term "apoptosis" is derived from the Greek words "απο" and "πτωσις", meaning "dropping off" and refers to the falling of leaves from trees in autumn.
Morphological changes in apoptosis

Introduction

Morphological alterations of apoptotic cell death that concern both the nucleus and the cytoplasm are remarkably similar across cell types and species.
Morphological hallmarks of apoptosis in the nucleus are:

- Chromatin condensation and nuclear fragmentation,
- Which are accompanied by:
  1) rounding up of the cell,
  2) reduction in cellular volume (pyknosis),
  3) retraction of pseudopodes.
Chromatin condensation starts at the periphery of the nuclear membrane, forming a crescent or ring-like structure.
The chromatin further condenses until it breaks up inside a cell with an intact membrane, a feature described as karyorrhexis, and the plasma membrane is intact throughout the total process.

Introduction
At the later stage of apoptosis some of the morphological features include:

1) membrane blebbing
2) ultrastructural modification of cytoplasmic organelle
3) and a loss of membrane integrity (1)
Biochemical changes in apoptosis

1) activation of caspases,

2) DNA and protein breakdown and

3) membrane changes and recognition by phagocytic cells
Early in apoptosis

here is expression of phosphatidylserine (PS) in the outer layers of the cell membrane which has been "flipped out" from the inner layers. This allows early recognition of dead cells by macrophages, resulting in phagocytosis without the release of pro-inflammatory cellular components.
This is followed by a characteristic breakdown of DNA into large 50 to 300 kilobase pieces.
Later there is internucleosomal cleavage of DNA into oligonucleosomes in multiples of 180 to 200 base pairs by Endonucleases(2)
Another specific feature of apoptosis is the activation of a group of enzymes belonging to the cysteine protease family named caspases.

The "c" of "caspase" refers to a cysteine protease, while the "aspase" refers to the enzyme's unique property to cleave after aspartic acid residues.
Activated caspases

1) cleave many vital cellular proteins

2) and break up the nuclear scaffold and cytoskeleton.

3) They also activate DNAase, which further degrade nuclear DNA.
Introduction

Mechanisms of apoptosis

Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners.

There are three pathways by which caspases can be activated.
Introduction

The two commonly described initiation pathways are the

1) **intrinsic** (or mitochondrial)

2) and **extrinsic** (or death receptor)

3) A third less well-known initiation pathway is the **intrinsic endoplasmic reticulum** pathway.
The **extrinsic** death receptor pathway
The extrinsic death receptor pathway

The best known death receptors is the type

1 TNF receptor (TNFR1)
and a related protein called Fas (CD95)
and their ligands are called TNF and Fas ligand (FasL) respectively
The extrinsic death receptor pathway

Introduction

These death receptors have an intracellular death domain that recruits adapter proteins such as TNF receptor-

2) associated death domain (TRADD) and

3) Fas-associated death domain (FADD), as well as cysteine proteases like caspase 8
the whole ligand-receptor-adaptor protein complex is known as

the death-inducing signalling complex (DISC)

DISC then initiates the assembly
and activation of pro-caspase 8

The extrinsic death receptor pathway
The **intrinsic** mitochondrial pathway
Internal stimuli such as irreparable genetic damage, hypoxia, extremely high concentrations of cytosolic Ca\(^{2+}\), and severe oxidative stress are some triggers of the initiation of the intrinsic mitochondrial pathway.
Regardless of the stimuli, this pathway is the result of increased mitochondrial permeability and the release of pro-apoptotic molecules such as cytochrome-c into the cytoplasm.
Introduction

This pathway is closely regulated by a group of proteins belonging to the Bcl-2 family, named after the BCL2 gene originally observed at the chromosomal breakpoint of the translocation of chromosome 18 to 14 in follicular non-Hodgkin lymphoma.
There are two main groups of the Bcl-2 proteins.

1) namely the pro-apoptotic proteins (e.g. Bax, Bak, Bad, Bcl-XBs, id, Bik, Bim and Hrk).

2) and the anti-apoptotic proteins (e.g. Bcl-2, Bcl-X_L, Bcl-W, Bfl-1 and Mcl-1).

The **intrinsic** mitochondrial pathway
Other apoptotic factors that are released from the mitochondrial intermembrane space into the cytoplasm include:

1. Apoptosis inducing factor (AIF)
2. Second mitochondria-derived activator of caspase (Smac)
3. Direct IAP Binding protein with Low pI (DIABLO)
4. Omi/high temperature requirement protein A (HtrA2)
The **intrinsic** mitochondrial pathway

Cytoplasmic release of cytochrome c activates caspase 3 via the formation of a complex known as **apoptosome** which is made up of:

1) cytochrome c
2) Apaf-1
3) and caspase 9
**Introduction**

The *intrinsic* mitochondrial pathway promotes caspase activation by binding to inhibitor of apoptosis proteins (IAPs), which subsequently leads to disruption in the interaction of IAPs with caspase-3 or -9.
It is believed to be caspase 12-dependent and mitochondria-independent. The intrinsic endoplasmic reticulum pathway is believed to be caspase 12-dependent and mitochondria-independent. When the ER is injured by cellular stresses like hypoxia, free radicals, or glucose starvation.
Introduction

Disrupted balance of pro-apoptotic and anti-apoptotic proteins

The Bcl-2 family of proteins (IAPs) and p53

Apoptosis and carcinogenesis

Reduced caspase activity

Impaired death receptor signaling (6)
Cancer therapy via The *extrinsic* death receptor pathway
RhApo2L/TRAIL effectively induces apoptosis in vitro in a broad spectrum of cancer cell lines, including lung, colon, pancreas, and lymphoma.
RhApo2L/TRAIL also has demonstrated single-agent activity in vivo in preclinical tumor models based on SCID, athymic nude mice bearing CRC, NSCLC, non-Hodgkin’s lymphoma (NHL) xenografts.
In the first of two studies, *cynomolgus* monkeys were administered *rhApo2L/TRAILE* at doses of.

Discussion

*Preclinical Safety of rhApo2L/TRAILE*

0, 0.1, 1.0, or 10 mg/kg/d

For

7 days
Neither detectable signs of liver toxicity nor changes in liver enzyme activity were observed. No evidence of toxicity to any other major organ system or tissue was detected. (6)
Activity of rhApo2L/TRAIL and rituximab

1) Mice (n = 12 mice/group) were then treated with vehicle (black x),

2) 60 mg/kg rhApo2L/TRAIL (closed blue circle),

3) 4 mg/kg rituximab (open green triangle),

4) 10 mg/kg rituximab (open red square),

5) rhApo2L/TRAIL and 4 mg/kg rituximab (closed green triangle),

6) or rhApo2L/TRAIL and 10 mg/kg rituximab (closed red square).
Cancer therapy via

The *intrinsic* mitochondrial pathway
Oxidative stress is a biochemical condition that is characterized by the imbalance between relatively high levels of toxic reactive species.
Discussion

Oxidative Stress

1) reactive oxygen species (ROS),

2) reactive nitrogen species (RNS),

3) and the antioxidative defense mechanisms. 1–3

ROS and RNS are organic or inorganic molecules that have an odd number of electrons
Oxidative Stress

http://www.in-corpore.ch/news/oxidative-stress-nutrition/
Last update:2017/04/14
The biological functions of oxidative stress and its potential role in cancer development and progression have been investigated for several decades. Cancer itself induces oxidative stress.
Discussion

actually

ROS levels have been found
to be significantly
higher and

Oxidative Stress

GPX and SOD activities
significantly
lower in cancer patients than
controls(7)
Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis


*State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, China, †Key Laboratory of Bioresources and Eco-environment, Ministry of Education, School of Life Sciences, Sichuan University, Chengdu, China, §School of Life Sciences, Guizhou Normal University, Guiyang, China, and ¶Shanghai Key Laboratory for Pharmaceutical Metabolite Research, School of Pharmacy, Second Military Medical University, Shanghai, China

Received 10 March 2012; revision accepted 9 July 2012

Abstract
Programmed cell death (PCD), referring to apoptosis, autophagy and programmed necrosis, is proposed to be death of a cell in any pathological format, when mediated by an intracellular program. These three forms of PCD may jointly decide the fate of cells of malignant neoplasms; apoptosis and

Introduction: a brief overview of programmed cell death

Programmed cell death (PCD) may balance cell death with survival of normal cells; the equilibrium becomes disturbed and PCD plays key roles in ultimate decisions of cancer cell fate (1,2). Of note, apoptosis, autophagy and programmed necrosis are the three main forms of
It is well established that some chemotherapeutic agents and radiation therapy
generate ROS in patients during cancer therapy
Chemotherapy agents can be divided into several categories:

1) alkylating agents (e.g., cyclophosphamide, ifosfamide)

2) anthracycline antibiotics which affect nucleic acids (e.g., doxorubicin, bleomycin)

3) platinum compounds (e.g., cisplatin)
BCL2 is known to prevent programmed cell death, increase metastatic potential, and promote resistance to anticancer therapy.
High expression of the BCL2 proto-oncogene is found in various solid tumours.

Discussion

G3139 is an antisense phosphorothioate oligodeoxynucleotide that suppresses BCL2 expression.
Bcl-2 Inhibitors: Targeting Mitochondrial Apoptotic Pathways in Cancer Therapy

Min H. Kang\textsuperscript{1,2} and C. Patrick Reynolds\textsuperscript{1,3,4}

\textsuperscript{1} Cancer Center and the Department of Cell Biology and Biochemistry, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas

\textsuperscript{2} Department of Pharmacology, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas

\textsuperscript{3} Department of Internal Medicine, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas

\textsuperscript{4} Department of Pediatrics, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas
bcl2
BCL2 antisense therapy is well tolerated

The first results of a phase I dose-escalation clinical trial evaluating subcutaneous administration of G3139(8)
One of the most advanced gene therapy agents used in the treatment of human cancer is the replication-incompetent adenovirus that delivers a \( p53 \) expression cassette, RPR/INGN201.
Preclinical studies in human cell lines and animals with head and neck cancers have shown that the $p53$ gene contained in RPR/INGN201 is efficiently transcribed and translated into p53 protein.
Discussion

Treatment with RPR/INGN201 inhibited cell growth in human SCCHN cell lines of diverse p53 status and suppressed tumour growth in animal xenografts of human SCCHN through the apoptotic pathway and other effects, such as inhibition of angiogenesis.\(^{(9)}\)
p53

https://pdb101.rcsb.org/motm/31
LAST UPDETED:12/04/2017
Control of apoptosis by p53

Jordan S Fridman\(^1,3\) and Scott W Lowe*\(^2\)

\(^1\)Incyte Corporation, Wilmington, DE 19880
\(^2\)Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA

The p53 tumor suppressor acts to integrate multiple stress signals into a series of diverse antiproliferative responses. One of the most important p53 functions is its ability to activate apoptosis, and disruption of this process can promote tumor progression and chemoresistance. p53 apparently promotes apoptosis through transcription-dependent and -independent mechanisms that act in concert to ensure that the cell death program proceeds most commonly inactivated tumor suppressor gene in human cancer (Hussain and Harris, 1998; Beroud and Soussi, 2003).

Although most of the attention on p53 has focused on its role in cancer, chronic activation of this key biological pathway may be equally as deleterious as its inactivation. In fact, hyperactivation of p53 has been associated with a variety of degenerative diseases such as...
caspase-8 is mutated in different types of cancers

Soung and colleagues screened gastric carcinomas (162 cases),

breast carcinomas (93 caspase),

non–small cell lung cancers (NSCLC) (185 cases),

and 88 acute leukaemias (88 cases)

for mutations within the caspase-8 gene using single strand conformation polymorphism (SSCP)
They found that the incidence of caspase-8 mutation in gastric cancer is statistically higher than those of NSCLC breast cancer, and acute leukaemias.

Furthermore, all of the 13 mutations detected were in advanced gastric cancers but not in early gastric cancers.
The mutations consisted of:

1) three missense,

2) one in-frame deletion, and

3) five frameshift mutations in the coding sequences;

4) two mutations in the initiation codon;

5) three mutations in the introns; and one mutation in the 3' untranslated region.
Pancreatic cancer is one of the leading causes of cancer related death in the world.

CASPASE FAMILY

1) Smoking,
2) diabetes mellitus history,
3) alcohol drinking

are risk factors for pancreatic carcinogenesis.
It has been shown that pancreatic cancer cells often present non-functional CD95/Fas and aberrant expression of FasL and this mechanism may contribute to the malignant and often rapid course of the disease.
Discussion

CASPASE FAMILY

caspase-3 was mutated in one case in

1) stomach adenocarcinoma

2) one case in lung cancer,

3) four cases in colon cancer,

4) one case in hepatocellular carcinoma,

5) and one case in multiple myeloma
The mutations consisted of:

1) six missense mutations,
2) four silent mutations,
3) two mutations in the introns,
4) one mutation in the 59-untranslated region,
5) and one mutation in the 39-untranslated region.
Caspase-9 is a virtually ubiquitous protease, constitutively expressed in a variety of fetal and adult human tissues.

Mutational analysis of caspase-9 was performed in neuroblastoma tissues.
They isolated genomic DNAs from normal and tumour tissues of the same patients. Silent mutations were detected in two colorectal carcinomas and one gastric carcinoma.
In a case–control study, lung cancer patients and age and gender matched healthy controls were investigated for caspase-9 promoter polymorphism in lung cancer.
They reported that the -21263 GG genotype was associated with a significantly decreased risk of lung cancer compared with the -21263 AA or the combined 21263 AA + AG genotype.
They found that caspase-9 polymorphisms were significantly associated with the risk of lung cancer in the smokers but not in the non-smokers, which reflects a gene–environment interaction.
Such an interaction is biologically plausible because smoking is a major risk factor for lung cancer. It was also found that the association between caspase-9 polymorphisms and the risk of lung cancer was statistically significant in the light smokers but not in the heavy smokers (10).
Most of what we know about apoptosis has been developed and understood only recently. The core idea of designing therapeutic drugs for cancer is based on the fact that worn out or damaged cells commit suicide in order for the body to continue to grow normally, maintaining a healthy number of cells, whereas this phenomenon is greatly disturbed in cancer cells.
we are still left with huge challenges to be overcome. Development of drugs that act either by blocking the action of antiapoptotic proteins, such as IAPs, small-molecule inhibitors (antisense oligonucleotides),
or by halting, hampering or interference with the transcription of RNA, such as small interfering RNA, BH3 mimetic and some HDACi, holds robust potential for use in cancer therapy; however, the hope to cure cancer is yet to be seen
References

1) **Apoptosis in cancer therapy**: crossing the threshold

2) **Apoptosis in cancer therapy**: crossing the threshold

3) **Targeting apoptosis pathways in cancer therapy**

4) **The role of apoptosis in cancer development and treatment response**

5) **Bax plays a pivotal role in thapsigargin-induced apoptosis of human colon cancer HCT116 cells by controlling Smac/Diablo and Omi/HtrA2 release from mitochondria**

6) **Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy**

7) **Oxidative stress in end-stage renal disease: an emerging threat to patient outcome**

8) **Control of mitochondrial apoptosis by the Bcl-2 family**

9) **The genetics of the p53 pathway, apoptosis and cancer therapy**

10) **… of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world**